Stability against brushing abrasion and the erosion-protective effect of different fluoride compounds

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Abstract: This study aimed to analyse the impact of brushing on the protective effect of different fluoride solutions on enamel and dentin erosion. Bovine enamel and dentin specimens were rinsed once with TiF4, AmF, SnF2 (0.5 M F, 2 min) or water (control). Specimens were either left unbrushed or brushed with 10, 20, 50, 100 or 500 brushing strokes in an automatic brushing machine (2 N, non-fluoridated toothpaste slurry). Ten specimens per group were eroded with hydrochloric acid (HCl) (pH 2.3) for 60 s, and calcium release into the acid was determined by atomic absorption spectroscopy. Additionally, enamel and dentin surfaces were analysed by X-ray energy-dispersive spectroscopy (EDS) (n = 6/group) and scanning electron microscopy (SEM) (n = 2/group) before brushing and after 500 brushing strokes. Statistical analysis ($p < 0.05$) was performed by three- and one-way ANOVA (calcium release) or repeated measures ANOVA (EDS). TiF4, AmF and SnF2 reduced the erosive calcium loss in unbrushed specimens to 58-67% (enamel) and 23-31% (dentin) of control. Calcium release increased with increasing brushing strokes prior to erosion and amounted to 70-88% (enamel) and 45-78% (dentin) of control after 500 brushing strokes. Brushing reduced the surface concentration of fluoride (AmF), tin (SnF2) and titanium (TiF4). SEM revealed that surface precipitates were affected by long-term brushing. Brushing reduced the protective potential of TiF4, AmF and SnF2 solutions. However, considering a small number of brushing strokes, the protective effect of fluoride solutions is only slightly affected by brushing abrasion.

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Stability against Brushing Abrasion and the Erosion-Protective Effect of Different Fluoride Compounds.

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None of the authors of the present paper has a conflict of interest.

Abstract

This study aimed to analyse the impact of brushing on the protective effect of different fluoride solutions on enamel and dentin erosion.

Bovine enamel and dentin specimens were rinsed once with TiF_4, AmF, SnF_2 (0.5 M F, 2 min) or water (control). Specimens were either left unbrushed or were brushed with 10, 20, 50, 100 or 500 brushing strokes in an automatic brushing machine (2 N, non-fluoridated toothpaste slurry). Ten specimens per group were eroded with HCl (pH 2.3) for 60 s, and calcium release into the acid was determined by atomic absorption spectroscopy. Additionally, enamel and dentin surfaces were analysed by X-ray energy-dispersive spectroscopy (EDS, n = 6/group) and scanning electron microscopy (SEM, n = 2/group) before brushing and after 500 brushing strokes. Statistical analysis ($p < 0.05$) was performed by three- and one-way ANOVA (calcium release) or repeated measures ANOVA (EDS).

TiF_4, AmF and SnF_2 reduced the erosive calcium loss in unbrushed specimens to 58 – 67% (enamel) and 23 – 31% (dentin) of control. Calcium release increased with increasing brushing strokes prior to erosion and amounted to 70 – 88% (enamel) and 45 - 78% (dentin) of control after 500 brushing strokes. Brushing reduced the surface concentration of fluoride (AmF), tin (SnF_2) and titanium (TiF_4). SEM revealed that surface precipitates were affected by long-term brushing.

Brushing reduced the protective potential of TiF_4, AmF and SnF_2 solutions. However, considering a small number of brushing strokes, the protective effect of fluoride solutions is only slightly affected by brushing abrasion.

Introduction

The efficacy of fluorides and fluoride-containing compounds to prevent dental erosion is increasingly studied in the last years. While initial studies concentrated mainly on fluoride compounds forming CaF_2 precipitates on the surface, current research focuses on the efficacy of fluoride compounds containing polyvalent metal ions, such as tin, tintanium or iron [Wiegand et al., 2008a; Schlueter et al., 2009c; Bueno et al., 2010]. The protective potential of the latter is referred to the formation of acid-resistant surface coatings and an incorporation of fluoride and the respective ion in the hydroxyapatite lattice [Schlueter et al., 2009b; Wiegand et al., 2010b]. In this context it was also shown that polyvalent cations have a considerable anti-erosive effect even in the absence of fluoride [Sales-Peres et al., 2007; Ganss et al., 2008].

Several studies demonstrated a wide range in the efficacy of different fluoride compounds to prevent enamel and dentin erosion [Ganss et al., 2008; Wiegand et al., 2009a; Schlueter et al., 2009a]. Depending on the kind of fluoride compound, the formation of the surface precipitates and, thus, the anti-erosive potential is related to the concentration and pH of the agent and the duration and frequency of application. While the acid resistance of fluoride precipitates was investigated in several studies [Attin et al., 2001; Ganss et al., 2007; Yu et al., 2010], only limited information about their abrasion resistance is available yet. This issue is of clinical interest, as dental hard tissues are usually exposed not only to erosive but also to abrasive influences, such as toothbrushing. Although toothbrushing with fluoridated toothpastes might reduce abrasion of eroded dental hard tissues compared to non-fluoridated toothpastes [Magalhaes et al., 2007; Magalhaes et al., 2008], the use of fluoridated toothpastes alone had only a limited beneficial effect on dental erosion when compared to high-concentrated fluoride agents [Ganss et al., 2004a; Lagerweij et al., 2006]. Thus, fluoride precipitates which do not resist abrasive forces to some extent would require either a frequent application of the agent or the use of vehicles with a high adherence to the tooth surface, such as fluoride varnishes.

In a recent study it was shown that the anti-erosive effects of different fluoride toothpastes were decreased as soon as the specimens were not only immersed in but brushed with the respective toothpaste slurries [Ganss et al., 2011]. This finding might be related to the limited physical resistance of either the fluoride precipitates formed on the surface or the eroded surface itself, in case that incorporation of fluoride or other ions in the softened layer does not lead to a complete rehardening [Wegehaupt et al., 2009; Ganss et al., 2011]. CaF_2 precipitates formed on enamel when sodium fluoride or amine fluoride is applied can be partially removed by brushing [Wegehaupt et al., 2009]. However, the ability of different fluoride compounds and complexes containing polyvalent metal cations to withstand brushing was not systematically anaylsed yet. Therefore, it was the aim of the present study to investigate the influence of brushing on the erosion-preventive effect of different fluoride solutions (AmF, TiF_4, SnF_2). The null hypothesis was that brushing will not increase the erosive calcium loss of enamel and dentin specimens treated with the different test solutions.

Methods

Sample preparation and allocation to the experiments

Cylindric enamel and dentin specimens (3 mm in diameter, in total 304 enamel and 304 dentin specimens) were obtained with a water-cooled trephine bur from the crowns or roots, respectively, of freshly extracted, non-damaged bovine incisors, which were stored in 0.9% NaCl solution until used. The samples were embedded in acrylic resin (Paladur, Heraeus Kulzer, Germany). Subsequently, enamel and dentin surfaces were ground flat and polished with water-cooled carborundum discs (1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Stuers, Erkrat, Germany) thereby removing approximately 200 µm of the outermost layer as verified with a micrometer (Digimatic, Mitutoyo, Tokyo, Japan).

Enamel and dentin specimens were randomly assigned to 4 groups (3 test: TiF_4, AmF, SnF_2, 1 control: water) of each n = 76 specimens. Sixty enamel and sixty dentin specimens were used for determination of erosive calcium loss and thus, were randomly assigned to 6

subgroups (n = 10) accordingly to the number of brushing strokes (0, 10, 20, 50, 100 or 500 brushing strokes) applied before erosion. The remaining enamel and dentin specimens (each n = 16) were used for scanning electron micorscopy (SEM) or x-ray energy dispersive spectroscopy (EDS), which were performed after application of the respective test solution (SEM: each n = 2, EDS: each n = 6) and after application of 500 brushing strokes (SEM: each n = 2, EDS: each n = 6).

The brushing machine used consisted of 6 brushing chambers for two specimens each, allowing for brushing of up to 12 specimens in parallel in one experimental run. In one experimental run, all specimens in the brushing machine were brushed with the same amount of brushing strokes. The sequence of experimental runs in terms of applied brushing strokes was randomly determined. Six pairs of specimens (1 pair = 2 specimens from the same subgroup) were randomly selected from the different subgroups, pretreated with the respective solution and randomly assigned to the brushing chambers.

Test solutions and brushing treatment

Enamel and dentin specimens were treated once with equimolar solutions (0.5 M F) of TiF_4, AmF, SnF_2 or with distilled water (control) for 2 min. The solutions were freshly prepared prior to application to the specimens. The pH of the solutions amounted to TiF_4: 1.3, AmF: 4.3 and SnF_2: 2.6 (Metrom 827 pH Lab, Metrom, Herisau, Switzerland). Specimens pretreated with TiF_4, AmF or SnF_2, were then rinsed for 10 s with distilled water.

Brushing of the specimens was performed in an automatic brushing machine as described previously [Wiegand et al., 2008b;Wiegand et al., 2009b]. The toothbrushes (Paro M43, Esro AG, Thalwil, Switzerland, 0.2 mm filament diameter) were applied at 2 N brushing force with a brushing frequency of 120 strokes/min. Each two specimens from the same subgroup were brushed in the same brushing well with 4 ml toothpaste slurry (2 ml/specimen). The non-fluoridated toothpaste slurry was prepared by mixing 100 g of a baseline formulation (saliva substitute (79.2%) [Göhring et al., 2004], 85% glycerine (10%), 1.62% sodium bicarbonate (10.3%), carboxymethylcellulose (0.5%)) with 20 g calcium pyrophosphate (Fluka, Buchs,

Switzerland, Lot: 88HO466). The RDA- and REA-value of the toothpaste slurry amounted to 50 and 6, resepctively, and was determined according to Imfeld [2010]. After brushing, the specimens were rinsed with distilled water for 10 s and gently air dried prior to erosion.

Erosive challenge and measurement of calcium loss

For determination of erosive calcium loss, the specimens (n = 10 per group) were eroded with hydrochloric acid (HCl, pH 2.3, 5.01 mmol/l) for 60 s after the respective brushing treatment. Each specimen was eroded in 1 ml HCl in an Eppendorf tube, which was gently shaken during sample incubation (60x/min). The amount of calcium dissolved from the enamel and dentin surfaces by erosive treatment was analysed by atomic absorption spectroscopy (Model 2380, Perkin-Elmer, Norwalk, CT) at 422.7 nm.

X-ray energy-dispersive spectroscopy and scanning electron microscopy

The surface composition of enamel and dentin specimens was obtained by X-ray energy-dispersive spectroscopy and scanning electron microscopy (SUPRA 50VP and Genesis, Carl Zeiss NTS GmbH, Oberkochen, Germany) directly after pretreatment with the test solutions and after 500 brushing strokes. Therefore, the specimens were desiccated for 4 weeks in blue silica gel [Schmidlin et al., 2001; 2002] in a vacuum evaporator. For EDS measurement a defined area of 200 x 200 µm was measured in secondary electron mode (15 kV, 100 s) with a penetration depth of approximately 3 µm. The weight percentage of the elements was analysed stoichiometrically.

For SEM examination specimens desiccated as described above, sputter-coated with gold for 60 s and the examined at 10 to 20 kV.

Statistical analysis

Calcium loss in enamel and dentin test groups was calculated as percentage of the mean calcium loss in the respective control group (rinsed with water, brushed with 0 – 500 brushing strokes). Normal distribution was checked with Kolmogorov-Smirnov and Shapiro-Wilk`s

tests. As normal distribution was found, the data were analysed by three-way ANOVA considering the kind of dental hard tissue, the number of brushing strokes or the test solution(s) as independent variable. Due to significant interaction, three-way ANOVA was followed by two-way ANOVA separately for enamel and dentin specimens [Neter et al., 1996]. Within each test group, one-way ANOVA followed by Scheffe`s post-hoc test was applied to analyse if the amount of calcium released by enamel or dentin specimens, respectively, increased with increasing brushing strokes.

Differences between test and control specimens within groups brushed with the same amount of brushing strokes were analysed by one-way ANOVA followed by Scheffe`s post hoc tests separately for enamel and dentin specimens.

Differences of the protective potential of the test solutions in enamel and dentin were analysed by unpaired means comparison.

The surface composition of the specimens before and after brushing (500 brushing strokes) was analysed by repeated measures ANOVA with Greenhouse-Geisser correction and independent samples tests after normal distribution (Kolmogorov-Smirnov test) was found in all groups.

SPSS (Version 16, IBM, Switzerland) was used for statistical analysis. Overall level of significance was set at $p \leq 0.05$.

Results

Calcium loss

Three-way ANOVA found the kind of dental hard tissue, the number of brushing strokes and the test solutions to be significant with respect to calcium loss. The interaction between all criteria was significant. Two-way ANOVAs showed both factors and the interaction to be significant with respect to dentin loss, while for enamel loss all factors but not the interaction were significant.

Erosive calcium losses (mean% of control, 95% CI) of enamel and dentin specimens are presented in Tables 1 and 2. Mean calcium loss of the control groups brushed with 0 to 500

brushing strokes varied between 27.5 – 33.1 nmol/mm^2 (enamel) and 22.9 – 25.9 nmol/mm^2 (dentin) and, thus, was stable over time.

Calcium release of enamel specimens, which were not brushed prior to erosion, was significantly reduced by AmF, TiF_4, SnF_2 to 58 – 67% of control. Brushing treatment reduced the protective effect of the test solutions, but the effect was significant for SnF_2 only. After 500 brushing strokes, the protective effect against enamel erosion was still significant for AmF and TiF_4.

In dentin, the test test solutions reduced calcium release of unbrushed samples significantly to 23 – 31% of control. In all groups, the protective effect of the test solutions decreased with increasing brushing strokes. Application of 500 brushing strokes increased calcium release to 45 - 78% of control. However, the erosion protective effect was still significant in all groups.

Generally, the protective effect of the test solutions was significantly higher in dentin compared to enamel specimens except for AmF at 100 and 500 brushing strokes. While brushing treatment affected the protective potential of the test solutions to various extents, this effect was generally higher in dentin than in enamel.

X-ray energy-dispersive spectroscopy

Element composition in the control specimens was not affected by brushing.

In enamel, brushing significantly reduced the surface fluoride concentration of specimens treated with AmF. In specimens tretated with SnF_2 and TiF_4 the concentration of tin or titanium, respectively, was also reduced, but this effect was not significant.

In dentin specimens, brushing reduced the surface fluoride concentration of all test groups. Also, the surface concentration of tin and titanium in specimens tretated with SnF_2 or TiF_4, respectively, was significantly reduced by brushing.

225 Scanning electron micrographs

226 The application of TiF_4 and AmF solutions resulted in the distinct formation of globular
227 surface precipitates on enamel and dentin, while SnF_2 application resulted in a structurally
228 modified surface without visible precipitation on the surface. Brushing clearly removed the
229 surface precipitates formed after TiF_4 and AmF application and altered the surface treated
230 with SnF_2 in a way, that enamel and dentin surfaces appeared quite similar after brushing
231 treatment.

232

233 **Discussion**

234 This in vitro study showed that the erosion-protective effect of AmF, TiF_4 and SnF_2 was
235 reduced by brushing over time, thereby, dentin was significantly more affected by abrasion
236 than enamel. As brushing decreased the erosion protective potential significantly in most
237 groups, the null hypothesis was rejected.

238 The present experiment was set up as screening procedure to evaluate to which extent the
239 erosion-preventive effect of the test solutions is affected by abrasion rather than as an
240 attempt to reflect clinical conditions. All test solutions were used at their native pH, as they
241 were shown to be most effective (higher surface fluoride concentration and better
242 incorporation of metal ions) at their native instead at buffered pH [Yu et al., 2010]. However,
243 it has to be taken into consideration that the low pH of the TiF_4 and SnF_2 solutions might
244 induce soft tissue irritations so that the use of the test solutions as an oral mouthrinse has to
245 be questioned.

246 Under clinical conditions, the presence of saliva and salivary pellicle influences the
247 dissolution and abrasion behaviour of enamel and dentin [Hall et al., 1999;Joiner et al., 2008]
248 as well as the formation and stability of fluoride precipitates [Ganss et al., 2007; Wiegand et
249 al., 2008a]. However, the results of a previous in vitro study indicate that the resistance of
250 CaF_2-precipitates against brushing abrasion is rather influenced by the presence of a pellicle.
251 Thereby, the stability of CaF_2-precipitates against brushing treatment was not significantly
252 different between specimens pretreated with artificial and human saliva [Wegehaupt et al.,

253 2009]. Moreover, the use of a pellicle-layer created in vitro by immersion in human saliva
254 seemed not reasonable, as the components of human saliva are rapidly altered or degraded
255 in vitro and might not reflect the bioadhesion process occuring in vivo [Hannig and Hannig,
256 2009].
257 Specimens were brushed in an automatic brushing machine which ensured a standardized
258 movement at a defined brushing force (2 N) [Wiegand and Attin, 2011]. Brushing was
259 performed with a fluoride-free toothpaste slurry reflecting the abrasivity of common
260 toothpastes to avoid additional fluoridation of the samples [Wegehaupt et al., 2009].
261 However, as oral hygiene is usually performed with fluoridated toothpastes, recharging of the
262 surface with fluoride might probably decrease the effect seen in the present study.
263 The number of brushing strokes applied was exaggerated to a total of 500 brushing strokes
264 to analyse the long-term physical resistance of surface precipitates and coatings. While the
265 application of 500 brushing strokes significantly decreased the erosion-protective potential in
266 most groups, the application of 10 to 20 brushing strokes – which is equivalent to the number
267 of brushing strokes each tooth might receive during in vivo toothbrushing – did not reduce
268 the efficacy of the test solutions significantly. This observation is in accordance with a
269 previous in situ study showing that the erosion-protective effect of TiF_4 and AmF on enamel
270 and dentin was only slightly reduced by additional brushing (30 s twice daily) of the samples
271 in a 3-day in situ erosion experiment [Wiegand et al., 2010a]. Moreover, the erosive calcium
272 loss of specimens treated with the AmF solution correspond to the results of Attin et al.
273 [2001] and Wegehaupt et al. [2009], where the stability of KOH-soluble fluoride against
274 brushing was tested. Brushing with 25 to 100 brushing strokes at 2.5 or 4 N brushing force,
275 respectively, decreased the amount of KOH-soluble fluoride formed after application of
276 sodium or amine fluoride (1% F) by 10-25%.
277 In the present study, the test solutions were almost equally effective to prevent enamel and
278 dentin erosion initally, but showed slight differences at the end of the experiment. Depending
279 on the time and frequency of application, fluoride compounds containing polyvalent metal
280 cations were often shown to have a higher protective potential on erosion – especially when

281 applied in cyclic erosion models without abrasion [Schlueter et al., 2009a; Schlueter et al.,
282 2009b; Ganss et al., 2010]. In this case, large amounts of the cation are incorporated in the
283 surface leading to a broad structurally modified and acid-resistant zone [Schlueter et al.,
284 2009b; Ganss et al., 2010]. As the test solutions were applied only once in the present
285 experiment, the surface precipitates and structural alterations are limited to the outermost
286 enamel or dentin surface, respectively. It can therefore be assumed that the abrasion stability
287 of dental hard tissues would be increased when the test solutions – at least those containing
288 polyvalent cations - were frequently applied.
289 Remarkably, the test solutions were more effective in dentin than in enamel, but fluoridated
290 dentin showed a higher susceptibility to abrasion compared to the enamel. Laboratory
291 experiments often revealed a higher protective efficacy of fluoride compounds on dentin than
292 on enamel, while in situ studies showed the opposite. Clinically, fluorides might be more
293 effective in enamel than in dentin, as the organic matrix influencing the efficacy of fluorides
294 might to some extent be affected by chemical and enzymatical degradation [Magalhaes et
295 al., 2011], which was not simulated in the present study.
296 One explanation for the observation that the surface precipitates were less resistant on
297 dentin than on enamel is the presence of the smear layer on dentin specimens, which was
298 not removed prior to application of the test solutions. Buchalla et al. [2007] have shown that
299 the presence of s smear layer on dentin surfaces do not hamper fluoride penetration into
300 dentin, but rather enhance fluoride uptake. From the results of the present study one can
301 speculate that at least parts of the precipitates are loosely bound to the dentin surface and
302 can be easily removed by brushing.
303 While the reaction of the fluoride ion with dentin is intensively studied [Ganss et al., 2004b;
304 Ganss et al., 2007; Bartlett et al., 2008], few is known about how metal ions, like tin or
305 titanium, interact with the different dentin components, especially with the organic matrix
306 [Ganss et al., 2010]. Thus, further research is necessary to investigate the binding
307 mechanisms of metal-containing fluoride compounds with dentin. However, although the

binding mechanism of the test compounds and adherence of the precipitates to the surface might be different, the test solutions were almost equally effective.

Within the limitations of this in vitro study it can be concluded that the test solutions (AmF, TiF_4, SnF_2) were able to prevent erosive calcium loss, while they were generally more effective on dentin than on enamel. Brushing prior to erosion decreased the protective efficacy of the test solutions by removing the surface precipitates, but the brushing effect was more pronounced on dentin compared to enamel. Considering a short brushing time with a small number of brushing strokes, the protective effect of the test solutions is only slightly affected.

Authors´ contributions

A.W. conceived and designed the experiments; S.S and B.S performed the experiments; M.R. and A.W. analyzed the data; A.W. and T.A. wrote the paper

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Legends

Table 1

Percentage of calcium release (mean% of control, 95% CI) in enamel groups

Within each row, groups that are significantly different from each other are marked by different capital letters. Within each column, groups that are significantly different are marked by different small letters.

Groups marked by * were not significantly different from the respective control.

Table 2

Percentage of calcium release (mean% of control, 95% CI) in dentin groups

Within each row, groups that are significantly different from each other are marked by different capital letters. Within each column, groups that are significantly different are marked by different small letters.

Groups marked by * were not significantly different from the respective control.

Table 3

Percentages of elements (mean ± standard deviation) on enamel surfaces after application of the test solutions (initial) and after brushing with 500 brushing strokes. The detection of silicium on brushed surfaces probably corresponds to remnants of the toothpaste abrasives.

In elements marked * the concentration was significantly changed by brushing compared to the initial concentration

Table 4

Percentages of elements (mean ± standard deviation) on dentin surfaces after application of the test solutions (initial) and after brushing with 500 brushing strokes. The detection of silicium on brushed surfaces probably corresponds to remnants of the toothpaste abrasives.

In elements marked * the concentration was significantly changed by brushing compared to the initial concentration

Figure 1

Representative scanning electron micrographs (60,000 magnification) of enamel surfaces treated with TiF_4 (a, b), AmF (c, d) and SnF_2 (e, f) after application of the test solutions (a, c, e) and after brushing with 500 brushing strokes (b, d, f).

Figure 2

Representative scanning electron micrographs (60,000 magnification) of dentin surfaces treated with TiF_4 (a, b), AmF (c, d) and SnF_2 (e, f) after application of the test solutions (a, c, e) and after brushing with 500 brushing strokes (b, d, f).

Group	Number of brushing strokes					
	0	10	20	50	100	500
TiF_4	61.7 [a] (50.8, 72.5)	69.0 [a,b] (60.8, 77.3)	61.6 [a] (55.9, 67.2)	67.9 [a] (59.7, 76.0)	66.2 [a] (59.1, 73.3)	69.9 [a] (61.4, 78.4)
AmF	58.1 [a] (49.7, 66.4)	54.3 [a] (44.7, 63.8)	51.5 [a] (40.7, 62.4)	51.6 [b] (47.5, 55.7)	58.8 [a] (44.5, 73.1)	70.1 [a] (63.3, 76.9)
SnF_2	67.3 [A,a] (56.9, 77.8)	74.3 [A,B,b] (64.7, 83.9)	77.0 [A,B,b] (68.2, 85.8)	77.9 [A,B,a] (69.4, 86.4)	85.1 [B,b] (79.7, 90.6)	87.9 [B,b,*] (77.6, 98.2)

Table 1

Group	Number of brushing strokes					
	0	10	20	50	100	500
TiF_4	31.0 ^{A,a} (25.2, 36.7)	31.5 ^{A,a} (27.1, 35.8)	32.6 ^{A,a} (29.5, 35.8)	32.1 ^{A,a} (28.6, 35.7)	34.0 ^{A,a} (29.4, 38.6)	44.6 ^{B,a} (38.5, 50.7)
AmF	23.3 ^{A,a} (21.3, 25.3)	27.8 ^{A,a,b} (24.6, 31.1)	33.2 ^{A,a} (27.0, 39.4)	28.8 ^{A,a} (23.9, 33.7)	53.5 ^{B,b} (45.6, 61.3)	77.6 ^{C,b} (62.1, 93.1)
SnF_2	23.8 ^{A,a} (18.4, 29.2)	23.7 ^{A,b} (18.0, 29.3)	24.7 ^{A,a} (18.2, 31.2)	31.3 ^{A,B,a} (24.8, 37.8)	46.7 ^{B,b} (36.7, 56.8)	62.6 ^{C,b} (54.1, 71.1)

Table 2

Group	Time point	Ca	P	O	F	Ti	Sn	Si
Control	initial	36.9 ± 2.2	20.3 ± 0.5	42.2 ± 1.9				
	after 500 BS	37.3 ± 2.0	20.4 ± 0.4	41.8 ± 2.5				0.5 ± 0.1
TiF_4	initial	33.7 ± 1.0	19.4 ± 0.2	44.0 ± 1.4	0.2 ± 0.3	2.5 ± 1.0		
	after 500 BS	33.7 ± 1.8	19.8 ± 0.3	44.1 ± 2.2	0.2 ± 0.2	1.7 ± 0.1		0.6 ± 0.1
AmF	initial	35.9 ± 2.3	18.5 ± 0.6	24.3 ± 2.0	21.3 ± 4.0			
	after 500 BS	36.9 ± 1.6	19.2 ± 2.4	37.2 ± 2.6*	5.1 ± 2.4*			0.5 ± 0.1
SnF_2	initial	38.2 ± 2.4	20.3 ± 0.5	38.9 ± 2.0	0.1 ± 0.1		1.9 ± 0.3	
	after 500 BS	37.3 ± 2.3	20.5 ± 0.5	40.5 ± 2.9	0.1 ± 0.1		1.2 ± 0.3	0.5 ± 0.1

Table 3

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Group	Time point	Ca	P	O	F	Ti	Sn	Si
Control	initial	34.6 ± 2.1	18.8 ± 0.7	46.5 ± 2.7				
	after 500 BS	34.7 ± 2.1	18.3 ± 0.9	46.5 ±2.8				0.5 ± 0.2
TiF_4	initial	24.2 ± 4.8	15.7 ± 1.6	51.2 ± 3.1	2.7 ± 0.9	6.3 ± 2.5		
	after 500 BS	29.6 ± 1.9*	16.1 ± 1.2	49.9 ± 1.7	1.1 ± 0.6*	1.2 ± 0.6*		2.1 ± 1.2
AmF	initial	34.1 ± 0.9	14.7 ± 1.6	25.3 ± 1.3	26.1 ± 1.7			
	after 500 BS	33.2 ± 0.7*	17.8 ± 0.6	48.3 ± 0.9*	0.3 ± 0.3*			0.5 ± 0.2
SnF_2	initial	31.4 ± 1.8	17.0 ± 1.2	43.6 ± 0.5	1.3 ± 0.5		6.8 ± 3.0	
	after 500 BS	33.9 ± 1.3	17.9 ± 0.7	44.8 ± 2.1	0.7 ± 0.3*		2.3 ± 0.6*	0.5 ± 0.2

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557 Table 4

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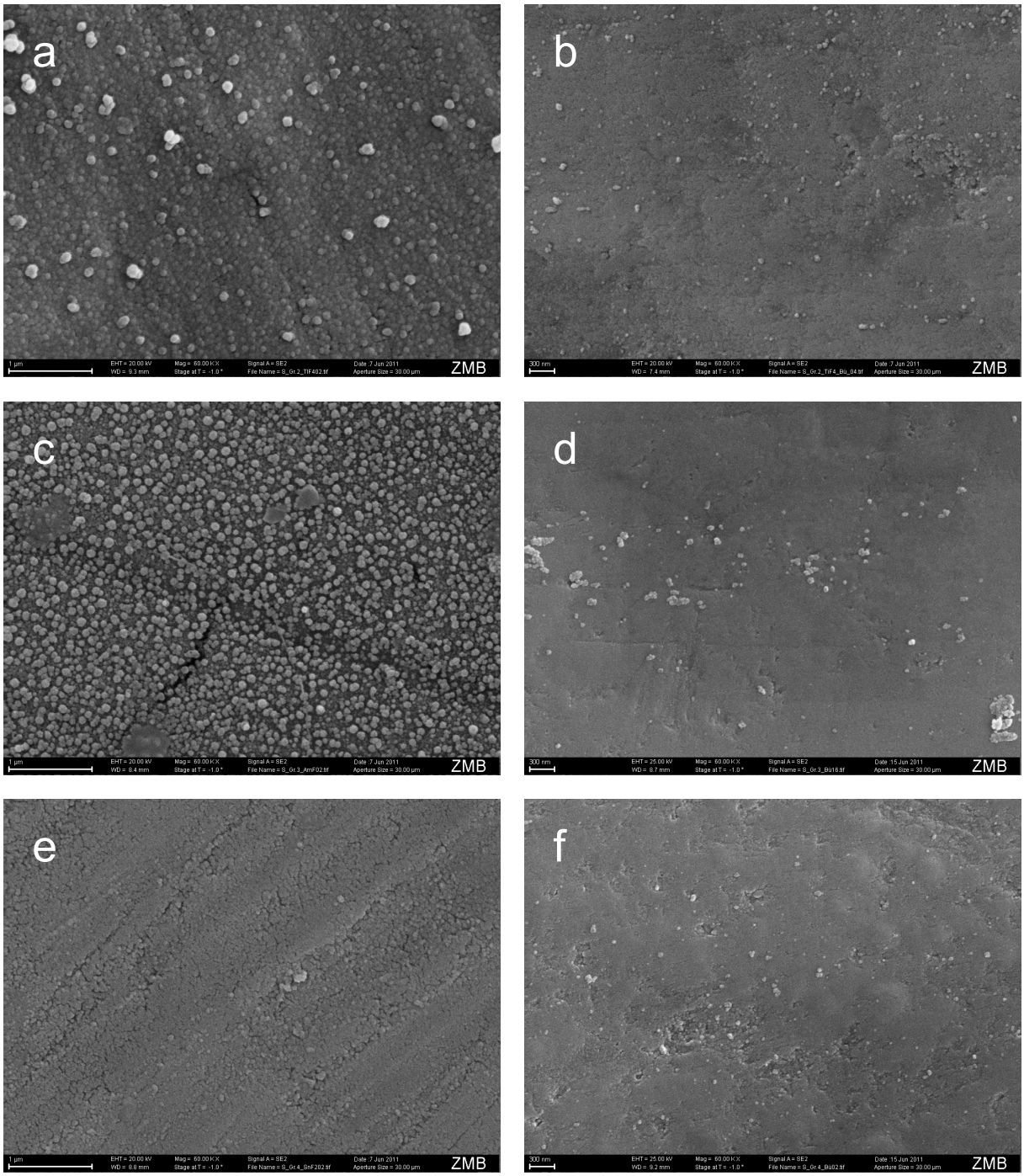

Figure 1

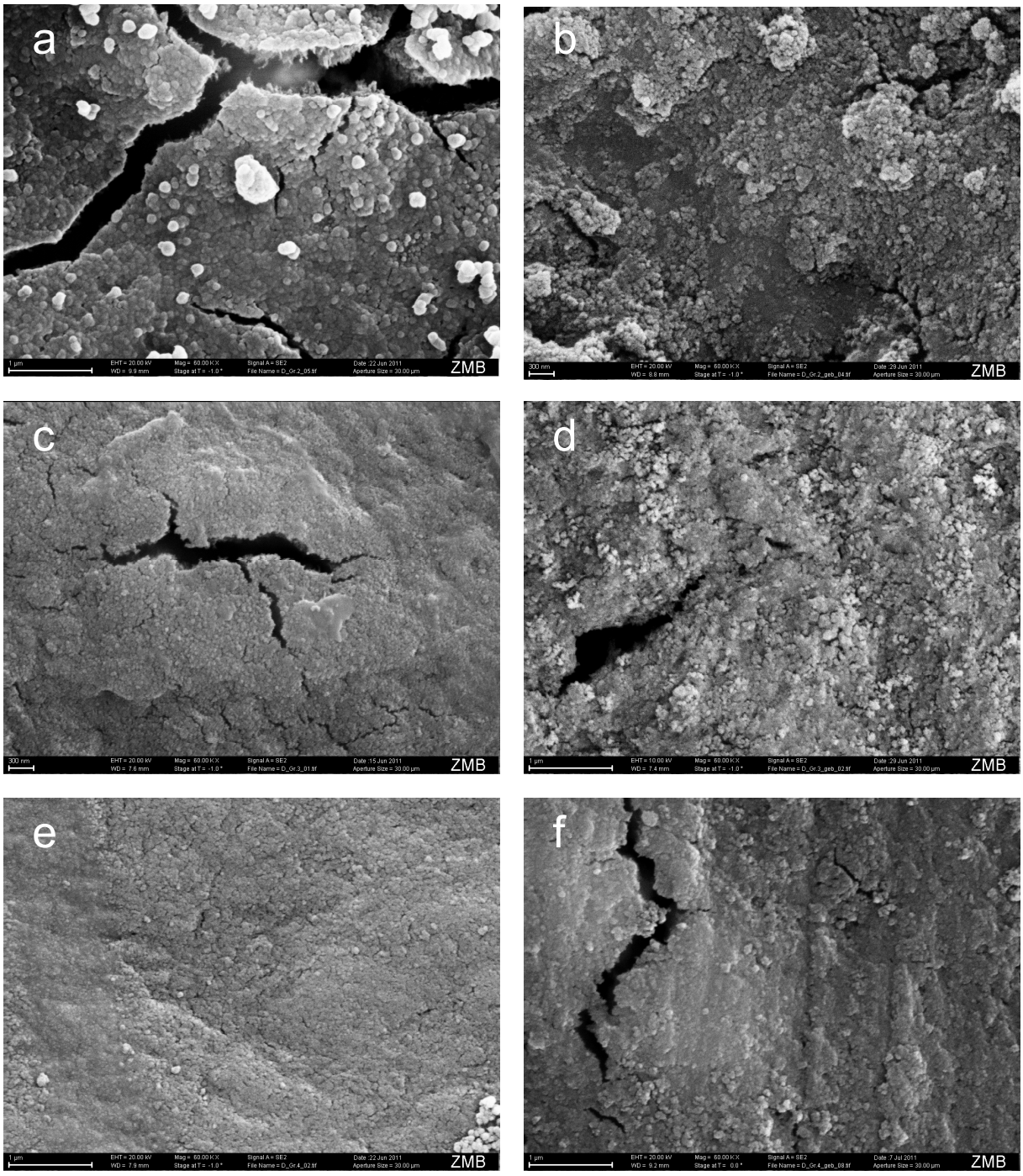

Figure 2